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Tracheal Reconstruction with End-to-end Anastomosis and Allografting: An Experimental Study

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ABSTRACT

Objective: We aimed to investigate the results of two tracheal reconstruction techniques (end-to-end anastomosis and tracheal allografting) in an experimental model.

Materials and Methods: Ten adult male New Zealand rabbits were used in the study. The animals were randomly divided into two groups of five, and underwent a tracheal resection of three segments. GroupI rabbits underwent an end-to-end anastomosis. The resected segments were transplanted to the groupII rabbits. The rabbits were sacrified at 20th postoperative day, and tracheal specimens were removed. The lateral and anteroposterior diameters of the lumen was measured, and the cross-sectional area (CSA) was calculated at the site of anastomosis. Specimens were investigated histopathologically for the inflammatory changes, fibrosis, and stenosis. The results were analysed statistically.

Results: The anastomosis was normal in every specimen. Secretions and fibrotic adhesions were insignificantly more common in the groupII, but the degree of fibrosis and inflammation was the same. In the groupII, there was one mild rejection. Both procedures caused a significant narrowing in the tracheal diameters ($p<0.05$). The CSAs reduced significantly in both groups (reduction to 84% and 82%, respectively, $p<0.05$). Therefore, these reductions were accepted as "normal" according to the proposed scale.

Conclusion: Both methods can be used in the tracheal reconstruction with an acceptable rate of stenosis.

Key words: *Allograft, animal experimentation, surgical anastomosis, trachea*

ÖZET

Uç Uca Anastomoz ve Allogreft ile Trakea Rekonstrüksiyonu: Deneysel Bir Çalışma

Amaç: Bu deneysel çalışmada iki farklı trakeal rekonstrüksiyon tekniğinin (uc uca anastomoz ve trakeal allogreft) sonuçlarının incelenmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmada 10 yetişkin Yeni Zelanda tavşanı kullanıldı. Hayvanlar beşerli iki gruba ayrıldı ve her birine üçer segmentlik trakea rezeksiyonu yapıldı. GrupI tavşanlara uc uca anastomoz uygulandı. Rezeke edilen segmentler ise GrupII tavşanlara allogreft şeklinde nakledildi. Tavşanlar postoperatif 20. günde sakrifiye edildiler ve trakeaları çıkarıldı. Lümenin yan ve ön-arka çapları ölçüldü ve anastomoz hattındaki kesit yüzey alanı hesaplandı. Örnekler inflamatuar değişiklikler, fibrozis ve stenozis açısından değerlendirilmek üzere histopatolojik incelemeden geçirildi. Sonuçlar istatistiksel olarak analiz edildi.

Bulgular: Her örnekte anastomoz hattı doğaldı. GrupII'de sekresyon ve fibrotik adezyon oranı istatistiksel olarak anlamsız şekilde daha sıktı, ancak fibrozis ve inflamasyon dereceleri her grupta aynıydı. GrupII'de bir örnekte hafif rejeksiyon vardı. Her iki teknik de trakeal çaplarda anlamlı daralma yaratmıştı ($p<0.05$). Kesit yüzey alanlar anlamlı şekilde her grupta azalmıştı (sırasıyla %84'e ve %82'ye düşüş, $p<0.05$). Ancak bu azalmalar önerilen ölçekle sınıflandırıldığında "normal" olarak kabul edilecek derecedeydiler.

Sonuç: Trakea rekonstrüksiyonunda her iki metod da kabul edilebilir stenoz oranıyla kullanılabilir.

Anahtar Sözcükler: *Allogreft, hayvan deneyi, cerrahi anastomoz, trakea*

Although the history of the tracheal and bronchial repair and anastomosis goes back to the late 19th century, the surgery of the trachea developed more slowly than the other areas of thoracic surgery, because of the anatomic complexities of the reconstruction and the biologic incompatibilities that met efforts at prosthetic reconstruction (1, 2). At midcentury, it was reported and believed that only 2cm or less of human trachea could be excised and still allow safe end-to-end anastomosis (3, 4).

After the development of the tracheal release manoeuvers, which provided the foundation of the modern tracheal surgery, it is now possible to resect more than 50% of the trachea and to obtain predictable healing by primary anastomosis (1, 5, 6). Yet, when extensive lesions are not amenable to primary end-to-end anastomosis, different methods and surgical techniques including solid and porous prostheses, the implantation of nonviable tissues, the usage of autogenous tissues, tissue engineering, and tracheal transplantation are used, as detailed in the review by Grillo (1). In the case of a tracheal reconstruction, tracheal stenosis is a major concern for thoracic and head and neck surgeons, due to complications in tracheal healing and repeated stenosis formation (7).

In this study we aimed to use two different methods of tracheal reconstruction (end-to-end anastomosis and tracheal

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allografting) and investigated the results of both techniques (micro-, and macroscopically) in an experimental rabbit model.

MATERIAL AND METHODS

This study was carried out in the Scientific Research Center of Fırat University Hospital, Elazığ, Turkey, from February to March 2009. All of the study protocols were approved by the Fırat University Ethics Committee on Animal Research. The animals were cared for in accordance with the Principles of Laboratory Animal Care of the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals formulated by the National Academy of Sciences (NIH publication 85-23, revised 1996).

Ten adult male New Zealand rabbits (aged 105 to 135 days, and weighing from 2800 to 3500g) were enrolled into this study. The animals were randomly divided into two groups of five each. The animals were kept in a wire top cage with free access to tap water. They were administered with standart food. Preoperatively each rabbit was kept off feed for a period of three hours before the induction of the anesthesia.

General anesthesia was obtained by intramuscular injection of 90mg/kg ketamine (Ketalar®, Pfizer, Turkey) and 10mg/kg Xylazine hydrochloride (Rompun®, Bayer, Turkey). Preoperatively the animals were given 50000 U/kg of intramuscular penicillin (Iecilline®, IE Ulagay, Turkey) as a primary prophylaxis to prevent any surgical infection. A line was maintained in one of the dorsal veins of the ear lobe through which mixture of serum solutions were passed if necessary. All the animals were operated under aseptic conditions, using spontaneous ventilation without an endotracheal tube. All the operations were performed by the same surgeon. Once asleep, the rabbit was fixed on a small table in supine position. The cervical area was shaved with a standart animal clipper. Then the surgical field was disinfected with povidone-iodine solution and draped in a standart fashion. A cervical longitudinal incision was performed, and after a thorough dissection of the subcutaneous and muscular planes, the trachea was exposed. Then the trachea was dissected and a segmental tracheal resection (5, 6, and 7th tracheal rings) was performed (Figure 1, and Figure 2).

End-to-end anastomoses were performed by using separated 5/0 silk sutures (Sterisilk®, SSM, Turkey) with full-thickness interrupted stitches all around the circumferential tracheal ring to the animals in the group I (Figure 3). The resected tracheal segments were used as fresh devascularized allografts. The excised allografts were immersed in a normal saline solution for not more than one hour, then they were transplanted to the animals in the group II. Anastomoses were performed by using the same suture material (Figure 4). Following an air-leak control using water, the incisions were closed with usual surgical methods.

Tramodol 1 mg/kg (Contramal®, Abdi Ibrahim, Turkey) and penicillin 50000 U/kg (Iecilline®, IE Ulagay, Turkey) were administered intramuscularly twice per day during seven postoperative days. The rabbits were housed in separated cages and were given a normal diet. Food and water intake and weights of the animals were monitored and recorded daily. The animals were sacrificed on the planned day (20th postoperative day) with an intraperitoneal injection of 100 mg/kg sodium penthotal (Thiopental sodium®, IE Ulagay, Turkey).

Tracheal specimens including the entire anastomosis site were removed. Each trachea was resected one centimeter far from the anastomotic line. The average measurement of the calibre of the trachea in each rabbit was calculated, measuring the lateral (a) and anteroposterior (b) diameters at each anastomotic level, and (c) the average measurement of 5mm proximal and distally away from the anastomoses (normal level). The cross-sectional area (CSA) was calculated as described (8): CSA= (a/2) x (b/2) x Π. The CSA measurement in the anastomotic level was called "anastomotic CSA", and the average of the CSA measured in the proximal and distal levels was called "normal CSA". Every "anastomotic CSA", and "normal CSA" values were compared in each group and the severity of the stenosis was noted according to the measurements of Hsieh et al (9): Very good: CSA 75-100%, Mild stenosis: CSA 50-74%, Important stenosis: CSA 30-49%, Critical stenosis: CSA 0-29%.

After the measurement, resected tracheal segments were taken to the Pathology Department of the Medical Faculty for further histopathological investigation. They were fixed with phormol at 10% and preserved in paraffin. In the macroscopic evaluation, any separation in the suture line, secretions in the luminal area, visible stenosis, and inflammatory changes were noted. Serial cuts were performed, stained with hematoxylin-eosin and viewed under a light microscope (NikonU-III multi-point sensor system, Japan), examining the wall integrity, the degree of inflammation in the mucosa, fibrosis, chondrogenesis, and foreign body reaction.

T-test was used for the comparison of numerical variables and the Fisher's exact test for the comparison of categorical variables. A p value of less than 0.05 was considered statistically significant.

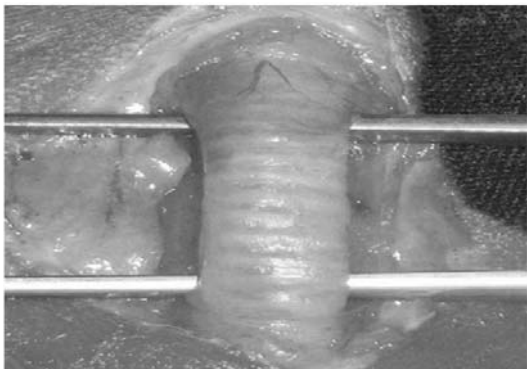

Figure 1. The exposition of the trachea.

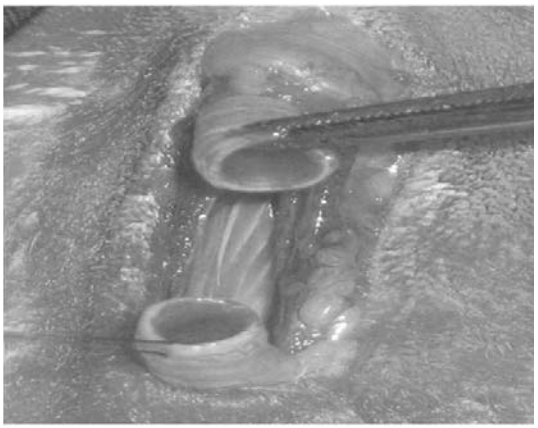

Figure 2. The view of the trachea after performing a three-segmental resection.

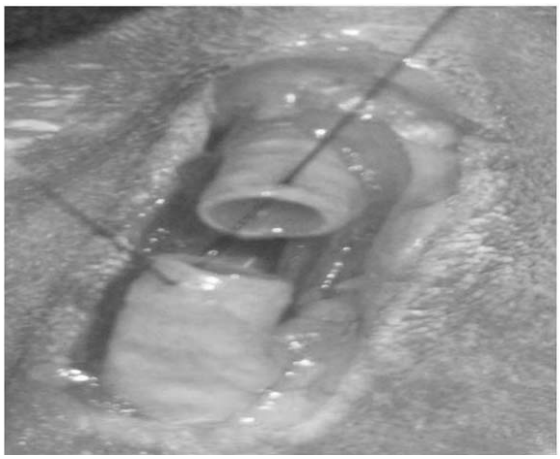

Figure 3. The end-to-end anastomosis procedure performed for the group I rabbits.

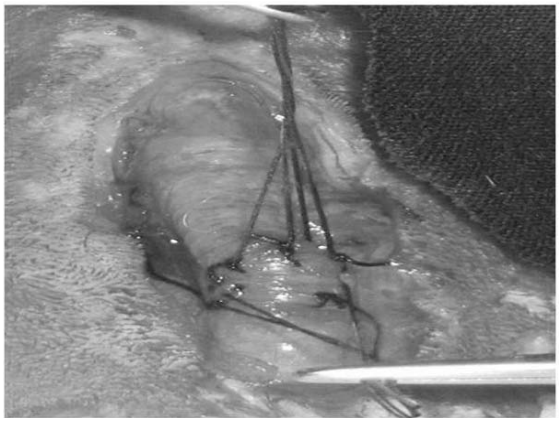

Figure 4. The transplantation procedure of the tracheal allograft for the group II rabbits.

RESULTS

There were no intraoperative complication. Except for one rabbit in group II who died on the third postoperative day, all of the animals survived until the day they were scheduled to be sacrificed. The postmortem pathological examination of the early deceased rabbit revealed that the lumen was filled with the secretions. The deceased rabbit was excluded from the analysis of the measurement of CSA of the airway, and from the analysis of the histopathological changes.

Histopathological changes. Macroscopically, the anastomotic line was normal in all specimens in both groups. Minimal intraluminal secretion was detected in one specimen in the group I, and two specimens in the group II ($p>0.05$). Fibrotic adhesions were detected in one rabbit in group I, and two rabbits in group II ($p>0.05$). Microscopically, there was no difference in the integrity of the tracheal wall, the chondrogenesis, and the epithelial metaplasia at different levels in all the rabbits. There was minimal edema and congestion in the mucosa, and moderate degree of fibrosis and inflammatory infiltration in each specimen. In the rabbits who underwent allografting, one showed (25%) a mild degree of rejection confirmed immunhistologically.

Measurement of the calibre of the airway. The measurements of the lateral and anteroposterior tracheal diameters in each group were given in Table 1 and Table 2. The measurements of the tracheal CSAs and the results in both groups were outlined in Table 3 and Table 4.

Both procedures resulted significant narrowing in the tracheal lateral and anteroposterior diameters. There was also a significant reduction in the CSAs after both procedures, and the actual airway opening ratio compared to normal measurement was 84% in the case of an end-to-end anastomosis, while it was 82% in the case of an allografting. Despite these reductions, the ratios were accepted as "causing no clinical stenosis", since it was reported that a ratio of more than 75% was "very good" in case of a tracheal anastomosis (9).

Table 1. Lateral tracheal diameters.

Rabbit number	Group I			Group II		
	A	N	A/N	A	N	A/N
1	5.6	6.1	0.91	5.7	6.0	0.95
2	5.7	6.3	0.90	5.6	6.1	0.91
3	6.0	6.3	0.95	5.8	6.2	0.93
4	5.6	6.0	0.93	5.5	6.0	0.91
5	5.7	6.2	0.91			

A: The measurement in the anastomotic line in mm

N: The measurement of the normal line in mm

A/N: The ratio of the measurement of the anastomotic and normal lines

Table 2. Anteroposterior tracheal diameters.

Rabbit number	Group I			Group II		
	A	N	A/N	A	N	A/N
1	5.1	5.5	0.92	4.8	5.5	0.87
2	5.1	5.5	0.92	5.0	5.5	0.90
3	5.2	5.7	0.91	4.8	5.4	0.88
4	4.9	5.4	0.90	4.7	5.4	0.87
5	4.8	5.4	0.88			

A: The measurement in the anastomotic line in mm

N: The measurement of the normal line in mm

A/N: The ratio of the measurement of the anastomotic and normal lines

Table 3. CSA measurement in both groups.

Rabbit number	Group I			Group II		
	A	N	A/N	A	N	A/N
1	22.34	26.25	0.85	21.40	25.82	0.82
2	22.74	27.11	0.83	21.91	26.25	0.83
3	24.41	28.09	0.86	21.78	26.19	0.83
4	21.47	25.35	0.84	20.22	25.35	0.79
5	21.40	26.19	0.81			

CSA: Cross-sectional area

A: The measurement in the anastomotic line in mm^2

N: The measurement of the normal line in mm^2

A/N: The ratio of the measurement of the anastomotic and normal lines

Table 4. The results of the end-to-end anastomosis and the tracheal allografting.

Group	Measurement	Anastomotic line	Normal line	Airway opening (%)	P value
I	Lateral	5.72±0.16	6.18±0.13	92	0.00119
I	Anteroposterior	5.02±0.16	5.50±0.12	91	0.00078
I	CSA	22.47±1.22	26.60±1.04	84	0.00043
II	Lateral	5.65±0.13	6.08 ± 0.10	93	0.00184
II	Anteroposterior	4.83±0.13	5.45±0.06	88	0.0001
II	CSA	21.33±0.77	25.90±0.41	82	<.0001

CSA: Cross-sectional area

DISCUSSION

Several tracheal pathologies including stenosis, neoplasm, trauma, postintubation injuries, and congenital diseases often require tracheal reconstruction. The concept of direct end-to-end anastomosis of trachea to trachea was generally accepted as the ideal method of trachea repair after reconstruction. For extensive lesions not amenable to primary and-to-end anastomosis, different methods and surgical techniques are used (1, 2). In the current study two methods (end-to-end anastomosis, and tracheal allografting) were used in an experimental animal model.

Anastomotic complications after tracheal resection and reconstruction are uncommon but lead to severe morbidity. These complications include granulations at the anastomotic line, stenosis, and separation (10). Stenosis of the trachea after tracheal reconstruction is mostly caused by excessive tension on the anastomosis, and this is related to overzealous resection of too great a length of trachea (2). Postoperative stenosis rate after the reconstruction via end-to-end anastomosis varies between 1.6 to 4.1% (11, 12). Fresh tracheal allografts were also demonstrated to result in fibrous stenosis (13). In our experimental study, it was clearly obvious that both techniques caused some significant degree of stenosis in each specimen. The CSA in the group of end-to-end anastomosis reduced to 84%, and to 82% in the group of tracheal allografting (p= 0.00043, and <.0001, respectively).

Not every stenosis causes clinical problem. It was reported by Hsieh et al (9) that when the CSA after the reconstruction is between 75 to 100%, the stenosis is called a "very good stenosis" and causes no clinical signs and symptoms. Myer-Cotton grading system for subglottic tracheal stenosis is another scale mostly used in children undergoing a laryngotracheal resection (14). According to this grading system, when the postoperative CSA is higher than 50%, it is termed as grade I stenosis, and requires no intervention. We basicly leaned on the classification proposed by Hsieh et al (9) in our study. We demonstrated that although there was a significant decrease in the postoperative CSA measurements in every specimens, this reduction in the calibre of the tracheal lumen was not clinically problematic, and thus was acceptable, according to both classification described above.

To prevent and reduce the degree of the postoperative tracheal stenosis, Olmos-Zúñiga et al (15) proposed the topical application of healing modulators such as hyaluronic acid or collagen polyvinylpyrrolidone in the tracheoplasty of a canine model. Thirty-three percent of the animals developed tracheal stenosis when the topical healing modulators were not applied. Dodge-Khatami et al (16) introduced the topical treatment of free tracheal autografts with vascular endothelial growth factor (VEGF) to diminish the degree of the stenosis. In their experimental study on the rabbits, the resected tracheal segments were soaked in either VEGF or normal saline solution. VEGF-trated rabbits demonstrated no stenosis while 33% of the others (normal saline solution group) developed a stenosis of less than 25% in the postoperative CSA measurement. We did not use any topical tissue healing modulators in the end-to-end anastomosis group, and immersed the allografts in a normal saline solution before the transplantation. As a result, there happened acceptable degrees of stenosis in every animals.

Another issue is the rejection following the tracheal transplantation. Fresh allografts require immunosuppressive therapy to counteract rejection (1). In an experimental using immunosuppressed pigs, Macchiarini et al (17) diagnosed mild rejections in 33% of the specimens after performing a heterotrophic tracheal transplantation. We did not use any immunsuppressive therapy for the rabbits in group II (allografting group). Only one (25%) rabbit showed an immunhistologically confirmed mild rejection.

In conclusion, the results of the current study clearly demonstrate that both methods, either end-to-end anastomosis or tracheal allografting, can be used in the resection and the reconstruction of the trachea with an acceptable rate of stenosis.

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